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Effects of Freezing on Toxicity of Sediments Contaminated with DDT and ENDRIN

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16. ABSTRACT

Two freshwater sediments containing 3 and 11% total organic carbon (TOC) were spiked with DDT and endrin to compare the effects of cold (4°C) and frozen (-20°C) storage of the sediment on toxicity to the amphipod *Hyalella azteca* in 10-d tests. Toxicity of the DDT-spiked sediment was reduced when it was frozen for 14 d. LC50 values for cold-stored and frozen-stored sediments were 4.2 and 7.3 µg/g DDT (calculated on dry solids basis) at 3% sediment TOC and 11.1 and 23.2 µg/g DDT at 11% sediment TOC, respectively. Toxicity of the 3% TOC endrin-spiked sediment was reduced by freezing; it exhibited LC50s of 5.1 and 7.7 µg/g endrin, respectively, for cold and frozen storage. Toxicity of endrin in two tests in the 11% TOC sediment remained essentially unchanged by freezing. LC50s were 19.6 and 21.7 µg/g endrin for cold and frozen sediments in one test and 10.3 and 9.8 µg/g endrin in the second test. Decreased toxicity was assumed to be related to the release of soluble organic carbon from the sediments during freezing and thawing. Relative differences in toxicity between DDT and endrin for cold and frozen sediments appear to be related to the different adsorptions of the chemicals onto the released carbon.

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EFFECTS OF FREEZING ON TOXICITY OF SEDIMENTS CONTAMINATED WITH DDT AND ENDRIN

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Abstract—Two freshwater sediments containing 3 and 11% total organic carbon (TOC) were spiked with DDT and endrin to compare the effects of cold (4°C) and frozen (−20°C) storage of the sediment on toxicity to the amphipod *Hyalella azteca* in 10-d tests. Toxicity of the DDT-spiked sediment was reduced when it was frozen for 14 d. LC50 values for cold-stored and frozen-stored sediments were 4.2 and 7.3 μg/g DDT (calculated on dry solids basis) at 3% sediment TOC and 11.1 and 23.2 μg/g DDT at 11% sediment TOC, respectively. Toxicity of the 3% TOC endrin-spiked sediment was reduced by freezing; it exhibited LC50s of 5.1 and 7.7 μg/g endrin, respectively, for cold and frozen storage. Toxicity of endrin in two tests in the 11% TOC sediment remained essentially unchanged by freezing. LC50s were 19.6 and 21.7 μg/g endrin for cold and frozen sediments in one test and 10.3 and 9.8 μg/g endrin in the second test. Decreased toxicity was assumed to be related to the release of soluble organic carbon from the sediments during freezing and thawing. Relative differences in toxicity between DDT and endrin for cold and frozen sediments appear to be related to the different adsorptions of the chemicals onto the released carbon.

Keywords—*Hyalella azteca* DDT Endrin Sediment toxicity Freezing

INTRODUCTION

The toxicity, persistence and bioavailability of organic compounds in sediment are important in evaluating their influence in aquatic ecosystems. Little information is readily available on how the toxicity and availability of sediment contaminants can be influenced by sample storage prior to analysis. Freezing of sediments is generally not recommended [1] but has been used as a preservation technique [2] and is still employed by some researchers and many state and federal pollution control agencies.

Contaminated estuarine sediment stored at 4 and −22°C for four weeks or more exhibited similar toxicities; however, the sediments at 4°C showed a general trend of increasing toxicity [3]. The effects of cold and frozen storage on a copper-spiked sediment have been studied in our laboratory by Malueg et al. [4], who demonstrated that freezing decreased the amount of soluble copper in the water, resulting in low or no toxicity to the cladoceran *Daphnia magna*. Total organic carbon (TOC) was higher in the water in contact with the frozen-stored as opposed to the cold-stored sediments, indicating a release of organic matter and a greater potential for the copper to occur in a complexed, less toxic form. There is much interest in the carbon normalization theory, which suggests that sediments with higher concentrations of TOC have a greater capacity to adsorb nonpolar organic compounds and thereby reduce their toxicity [5].

The objective of the present research was to evaluate the effect of freezing versus cold storage on the toxicity of DDT- and endrin-spiked sediments to the amphipod crustacean *Hyalella azteca*.

MATERIALS AND METHODS

Sediment

The two spiked sediments used in this experiment were collected from Soap Creek Pond No. 7, a 0.2-ha pond located on Oregon State University land 12 km north of Corvallis, Oregon, and from Mercer Lake, a 145-ha lake located 8 km north of Florence, Oregon. The Soap Creek Pond sediment was collected at a depth of 3 m with an Ekman grab sampler and maintained for several months in the bottom of outdoor 75-liter aquaria supplied with flowing well water (10–17°C). The Mercer

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Lake sediment was collected with Ekman and Ponar grab samplers at a depth of 10 m, placed in large polyethylene bags in ice chests and refrigerated at 4°C for up to nine months. Overlying water in all samples was decanted during collection. The Soap Creek Pond sediment contained 3% TOC and 23% dry solids and the Mercer Lake sediment contained 11% TOC and 15% dry solids (measured values).

The sediments were spiked with different concentrations of pesticide by partially coating flasks with DDT or endrin and then shaking them [6]. Each pesticide was dissolved in ethyl acetate prior to coating the inside of 2-liter Erlenmeyer flasks with a rotary evaporator. Approximately 1.6 to 1.8 kg wet sediment was added to each flask and was shaken at 150 oscillations per minute at room temperature for 7 d. Some chemical still adhered to the inside of the flasks after this time and was visible through the flask walls. The DDT-spiked and endrin-spiked sediments were then transferred to clean Erlenmeyer flasks and kept on a shaker for about 42 and 62 d, respectively, when most of it was used in another study [6]. The remaining spiked sediment was placed in storage at 4°C. The elapsed time between spiking and the beginning of the freezing experiments averaged about 9 months for the endrin-spiked sediments and about 19 months for the DDT-spiked sediments from Soap Creek Pond No. 7. Sediment from Mercer Lake was spiked with DDT at a later time, about 1.2 months before it was frozen. Pesticide concentration was measured at the beginning of the freezing experiments for all cold-stored and frozen-stored sediments. The amount of DDT or endrin in the sediment necessary to obtain the desired series of concentrations in the water for estimating 10-d LC50 values (lethal concentration killing 50% of animals in 10 d) was determined from previous range-finding tests and ranged from 1.3 to 195 μg DDT and from 1.3 to 267 μg endrin per gram dry solids (Table 1). Control sediments were prepared and shaken in the same manner as test sediments, but without the addition of DDT or endrin.

One hundred milliliters of test sediment at each concentration was poured into each of four replicate 1,000-ml beakers. The tops of the beakers were covered for storage with a layer of aluminum foil and further sealed by a layer of parafilm, all secured with a rubber band. One-half of the beakers (two per concentration) were returned to cold storage at 4°C and the other half were placed in a deep-freeze at −20°C for 14 d.

Water

The test water was obtained from a laboratory well near the Willamette River at Corvallis, Oregon. This water has low but variable levels of dissolved constituents and is characteristically soft [7]. To maintain uniform quality during the test period, the water was adjusted before testing to a total hardness of 200 mg/L as $CaCO_3$ by the addition of $CaSO_4 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$ and $NaHCO_3$ in a modification of a standard method [8]. After contact with the sediment, dissolved oxygen and pH in the test water ranged from 6.1 to 9.3 mg/L and from 6.1 to 7.9, respectively. Total hardness, alkalinity and conductivity all decreased once the water was added to the beakers, with ranges in all tests (from an initial high on day 0 to a low on day 10) of from 200 to 86 mg/L, from 163 to 30 mg/L and from 682 to 450 μS, respectively.

Test organisms

Hyalella azteca (Saussure) were cultured in flowing well water at 20°C on a 16-h light:8-h dark cycle. Pelleted DDT- and endrin-free (<0.1 μg/g) rabbit food, dry-blended to reduce particle size, was used as the main food source and substrate, and was fed to the *Hyalella* as required. Animals used for testing were about two months old. The largest juveniles/adults (4 mm long) that passed through a 1-mm standard sieve screen were collected for the tests. They were placed into holding containers to observe for injured animals a few hours before they were added to the test beakers. Each test beaker received 40 mg of powdered pelleted rabbit food on days 3 and 7 of the test. The delay in feeding until the third day of testing forced *Hyalella* to forage in the sediment, giving the organisms greater contact with potential toxicants.

Test conditions

The toxicity tests were conducted in duplicate for both the cold-stored and frozen-stored sediments. There was insufficient sediment available for more replications. All testing was done in ventilated Plexiglas cabinets in an environmental room at 20 ± 0.5°C under a 16-h light:8-h dark light regime, giving about 430 lux at bench top. Test procedures were generally those of Nebeker et al. [9,10], with modification for smaller volumes of test sediment.

When the beakers containing the pesticide-spiked sediment were removed from cold or frozen storage after 14 d, they were held at 20°C for 4 h

Table 1. Mortality of *Hyalella azteca* in 10-d tests using cold-stored and frozen-stored sediments spiked with DDT or endrin

Test	µg added/g dry solids[a]	Initial measured sediment concn. (µg/g dry solids)	Water concn.[b] (µg/L) Total Cold	Total Frozen	Soluble Cold	Soluble Frozen	No. *Hyalella azteca* dead (*n* = 20) Cold Rep. 1	Cold Rep. 2	Frozen Rep. 1	Frozen Rep. 2
DDT										
Soap Creek	0	<0.4	<0.1	<0.1	<0.1	<0.1	2	4	1	0
Pond No. 7	21.3	9.6	<1.1	<0.2	<0.1	<0.1	19	20	12	11
	47.8	16.1	1.1(0.5)	<0.4	<0.2	<0.1	20	20	18	17
	91.3	44.8	1.2(0.1)	0.7(0.2)	0.5(0.03)	0.4(0.1)	20	20	20	20
	173.9	83.5	2.5(1.4)	1.3(0.2)	0.9(0.01)	0.8(0.2)	20	20	20	20
	352.2	194.8	3.5(1.9)	1.8(0.2)	1.4(0.1)	1.1(0.2)	20	20	20	20
Soap Creek	0	<0.4	<0.1	<0.1	<0.1	<0.1	1	0	0	0
Pond No. 7	–[c]	1.8	<0.2	<0.1	<0.1	<0.2	0	1	0	1
	21.3	9.6	0.4(0.1)	<0.4	<0.2	<0.3	19	20	12	9
	47.8	16.1	0.3(0.04)	<0.3	<0.3	<0.3	20	20	17	19
	91.3	44.8	1.0(0.2)	0.6(0.1)	0.5(0.2)	0.5(0.1)	20	20	20	20
	173.9	83.5	1.3(0.3)	1.0[d]	0.8(0.2)	0.9(0.03)	20	20	20	20
Mercer Lake	0	<0.7	<0.1	<0.1	<0.1	<0.1	0	1	0	1
	13.3	1.3	<0.2	<0.2	<0.2	<0.2	0	1	2	0
	33.3	8.0	<0.5	<0.2	<0.2	<0.2	3	2	2	0
	66.7	20.0	<0.1	<0.2	<0.2	<0.2	19	20	7	10
	140.0	71.3	<2.5	<0.4	<0.5	0.2(0.1)	20	20	20	20
	280.0	160.0	2.9(1.6)	0.7(0.1)	0.6(0.1)	0.5(0.1)	20	20	20	20
Endrin										
Soap Creek	0	<0.4	<0.1	<0.1	<0.1	<0.1	2	1	1	2
Pond No. 7	13.0	3.0	1.4(0.1)	1.8(1.0)	1.1(0.3)	1.7(0.5)	1	5	2	1
	26.1	8.7	4.5(1.5)	4.1(0.6)	3.1(0.9)	4.1(1.3)	18	16	11	10
	47.8	19.6	7.2(1.9)	7.1(1.3)	6.1(1.5)	6.5(1.5)	20	20	20	20
	108.7	40.4	15.8(3.1)	20.6(5.2)	13.9(4.6)	18.1(4.9)	20	20	20	20
	200.0	62.1	27.9(6.0)	28.5(6.8)	22.2(5.2)	23.2(5.4)	20	20	20	20
Mercer Lake	0	<0.7	<0.1	<0.1	<0.1[e]	<0.1[e]	1	–	0	–
	13.3	2.0	0.5(0.2)	0.3(0.1)	0.4	0.5	2	–	1	–
	26.7	5.3	1.2(0.3)	1.1(0.2)	1.0	1.2	1	–	1	–
	46.7	13.3	2.0(0.5)	2.0(0.4)	2.4	2.4	5	–	4	–
	80.0	13.3	4.2(1.1)	2.9(0.6)	3.2	3.4	9	–	8	–
	153.0	–	11.1(3.0)	8.8(1.3)	10.9	9.9	20	–	20	–
	326.7	100.0	15.4(3.1)	17.2(2.2)	20.1	21.0	20	–	20	–
	606.7	266.7	52.6(10.0)	49.4(5.6)	65.0	–	20	–	20	–
Mercer Lake	0	<0.7	<0.1	<0.1	<0.1	<0.1	1	2	0	2
	6.7	1.3	0.5(0.1)	0.2(1.0)	0.3(0.1)	0.3(0.1)	0	1	0	1
	20.0	1.3	0.4(0.1)	0.3(0.1)	0.2(0.1)	0.4(0.1)	2	3	0	2
	40.0	8.0	2.0(0.6)	1.5(0.4)	0.8(0.3)	1.4(0.3)	3	1	3	5
	86.7	20.0	4.5(1.1)	3.9(0.7)	3.9(0.7)	3.7(0.7)	20	20	18	20
	160.0	66.7	12.3(2.2)	11.9(1.9)	10.8(2.1)	10.8(1.4)	20	20	20	20

[a]Amount coated on sides of flask in which wet sediment was spiked; not all chemical was adsorbed by the sediment.
[b]Mean (±SE) for days 0, 5 and 10.
[c]Prepared from 1:5 dilution of next highest concentration.
[d]Day 5 concentration only.
[e]Soluble endrin measured on day 10 only.

to warm or thaw. Each 100 ml of sediment was then stirred to mix the separated water and solids before 900 ml of 200 mg/L (as $CaCO_3$) hardness water was carefully added, using a baffle to minimize disturbance of the sediment bed. The beakers were allowed to stand for 1 d before the animals were added to allow particulates to settle and the water and sediment to interact. On day 0, gentle

aeration (using a 1 mm i.d. glass-tube-tipped plastic air line submerged so the tip was half-way between the water and sediment surfaces) was started a few hours before addition of animals. Twenty *Hyalella* were then added to each beaker. Mortality was determined at the end of 10 d to determine LC50 values for the test chemical in the cold and frozen sediment beakers. The organisms were isolated by pouring the contents of each beaker through a 0.59-mm mesh sieve screen and gently rinsing with water.

Two DDT tests and one endrin test were run using the 3% TOC Soap Creek Pond sediment; one DDT test and two endrin tests were run using the 11% TOC Mercer Lake sediment. The first Mercer Lake test with endrin was a screening test and so was not run with replicate beakers as were the others; however, it generated usable data.

Analytical procedures

Total pesticide was analyzed from a pooled water sample of 100 ml (50 ml from each replicate beaker); a similar 100-ml sample was analyzed for soluble pesticide. Volume was maintained in the beakers by replacement with hardness-adjusted well water on days 0 and 5. The pooled samples of water (100 ml) were extracted for DDT and endrin with ethyl acetate by liquid–liquid extraction with a magnetic stirrer [11,12]. The extracts were concentrated using a Kuderna-Danish concentrator [13]. Samples were then analyzed using fused silica capillary column GC with electron capture detection on a Hewlett-Packard 5730 gas chromatograph operated in the split mode at a split ratio of 50 to 75:1. Sediment samples were extracted with ethyl acetate:acetone (1:1, v/v) in a serum bottle and analyzed as above. Approximately 10% of the water samples were run in duplicate. Five or six standards were analyzed at the beginning of an analytical run, with a single standard analyzed after approximately every six samples. Prior to this experiment, the U.S. Environmental Protection Agency (EPA) QC sample WP385 was analyzed for *p,p'*-DDT. Recovery was 95% of the standard's reported value of 10 mg/L, with a coefficient of variation of 2% ($N = 6$).

The detection limits for DDT in water and sediment were 0.1 μg/L and 0.1 μg/g, respectively. The specific recovery of DDT in water based on extraction and injection was 103.9 ± 2.0% (mean ± SE, $N = 32$). The specific recovery of DDT in the sediment was 99.8 ± 2.0% (mean ± SE, $N = 4$). The mean coefficients of variation for water and sediment sample duplicate pairs were 10.3% ($N = 17$ pairs) and 20.0% for 22 sets of two to four replicates each. The detection limits for endrin in water and sediment were 0.1 μg/L and 0.1 μg/g, respectively. The specific recovery of endrin in the test water based on extraction and injection was 100.9 ± 1.3% (mean ± SE, $N = 12$). The specific recovery of endrin in the test sediment was 97.8 ± 4.5% (mean ± SE, $N = 2$). The mean coefficients of variation for water and sediment sample duplicate pairs were 4.3% ($N = 12$ pairs) and 6.6% for 12 sets of two to three replicates each.

Water quality was characterized by chemical analysis prior to and during testing. Dissolved oxygen and pH in the test beakers were measured daily by electrode. Total hardness, alkalinity and conductivity were determined on days 0 and 10 by U.S. EPA methods (Nos. 130.2, 310.1 and 120.1, respectively) [14]. Total carbon in the sediment was determined by combustion and measurement of carbon dioxide with a Coulometrics CO_2 coulometer [15]. Total sediment inorganic carbon was determined by a modification of the calcimeter method of Allison and Moodie [16]. TOC in the sediment was the difference between the total carbon and inorganic carbon values. Total and soluble organic carbon in the water was determined by coulometric detection [17]. Dry solids content of the sediment was determined by U.S. EPA method 160.3 [14]. Turbidity was measured with a Hach 18900 ratio turbidimeter on days 0, 5 and 10.

Samples for soluble DDT, endrin and organic carbon were centrifuged in 50-ml stainless steel tubes for 30 min at 16,000 rpm (30,900 *g*). The particulate-free water was decanted from precipitated settled solids. The stainless steel tubes and all glassware were washed with detergent, rinsed in tap and reverse-osmosis water, and baked overnight at 250°C before use.

Calculations

Mortality data from the two replicates for each concentration were pooled prior to calculation of the LC50s and their 95% confidence intervals by the trimmed Spearman-Karber method, in addition to calculating an LC50 value for each set of replicates [18]. Differences in mean LC50 values between cold and frozen tests were tested using a *t* test. Differences between soluble organic carbon values and differences between turbidity values were also compared with a *t* distribution. Relationships between pesticide concentration and turbidity were compared with the Pearson product moment coefficient of correlation. Mean DDT and endrin values for column water were obtained by averag-

ing the values observed on days 0, 5 and 10 of the tests.

RESULTS AND DISCUSSION

Freezing severely altered the physical appearance of the sediments: Before freezing, they were semigelatinous; after freezing, the sediments had clumped into coarse and chunky particles and had settled, losing about 25% in volume. Malueg et al. [4] also noted a similar decrease in sediment volume after freezing.

Turbidity in the beakers containing cold-stored sediments, due primarily to *Hyalella* foraging activities, averaged 97 NTU (1.6 to 890 NTU). Values were significantly less ($p < 0.05$) in the beakers containing frozen-stored sediment and averaged 9 NTU (0.6–69 NTU). The clumped sediment chunks in the frozen-sediment beakers were less able to be suspended by the foraging activities of the *Hyalella* than were the unaltered sediment particles in the cold-stored sediment beakers.

Mean total DDT averaged more than 100% higher in the water in beakers containing cold-stored sediments than in beakers with frozen-stored sediment; mean total endrin averaged only about 20% higher in the water from cold-stored sediment (Table 1). The higher values were probably related to the higher turbidities in the cold-stored sediment beakers. Correlation analyses between turbidity and pesticide concentration indicated r values of 0.69 for the cold-storage tests and only 0.04 for the frozen-storage tests. Soluble DDT and soluble endrin levels were similar between cold-stored and frozen-stored sediment beakers (Table 1).

Pooled mortality data from the two replicate sets were used in the LC50 calculations to present a single LC50 value and 95% confidence interval for all the organisms used in a test (Table 2). When LC50s were computed separately for each of the replicate sets in a test, the mean values were virtually identical with the pooled values.

Sediment LC50s in the first DDT test with Soap Creek Pond sediment were not calculable because of high mortality in the lowest concentrations in both the cold- and frozen-stored sediment tests (Table 2). Decreased toxicity in the frozen-stored sediments was evident, however, as indicated by the

Table 2. Ten-day water and sediment LC50 values in *Hyalella azteca* tests using cold- and frozen-stored DDT- and endrin-spiked sediments

	LC50 values (95% C.I.)[b]					
	Sediment concn. (μg/g dry solids)		Mean water concn. (μg/L; $n = 3$)			
			Total		Soluble	
Test[a]	Cold	Frozen	Cold	Frozen	Cold	Frozen
DDT						
Soap Creek Pond No. 7	<9.6	<9.6	–[c]	–[c]	–[c]	–[c]
Soap Creek Pond No. 7[d]	4.2 (3.9–4.4)	7.3 (6.0–9.0)	–[c]	–[c]	–[c]	–[c]
Mercer Lake[d]	11.1 (9.5–13.1)	23.2 (19.5–28.2)	–[c]	–[c]	–[c]	–[c]
Endrin						
Soap Creek Pond No. 7[d]	5.1 (4.5–5.8)	7.7 (6.4–9.3)	2.5 (2.2–2.9)	3.7 (3.2–4.2)	1.8 (1.6–2.1)	3.6 (3.1–4.1)
Mercer Lake	19.6 (14.5–26.5)	21.7 (16.6–28.4)	4.0 (3.0–5.3)	3.4 (2.8–4.2)	3.6 (2.8–4.7)	3.9 (2.2–4.8)
Mercer Lake	10.3 (8.6–12.3)	9.8 (8.1–12.1)	2.5 (2.2–2.9)	1.8 (1.5–2.2)	1.4 (1.2–1.7)	1.8 (1.5–2.1)

[a]Soap Creek Pond No. 7 sediment total organic carbon = 3%. Mercer Lake sediment total organic carbon = 11%. All analyses based on dry solids.
[b]LC50s based on pooled mortality data from the two sets of replicates.
[c]LC50 not calculable because some water concentrations were less than detection values.
[d]Mean LC50 values calculated from the two sets of replicate breakers were significantly different between cold and frozen tests ($p < 0.05$).

lower mortalities at DDT concentrations of 9.6 and 16.1 μg/g dry solids (Table 1).

In the second DDT Soap Creek Pond test, conducted 45 d later with the same sediments as the first test (but with an additional lower concentration prepared later), toxicity in the frozen-stored sediments also decreased. Here, an LC50 of 4.2 μg/g was obtained in the cold-stored sediment in contrast to an LC50 of 7.3 μg/g in the frozen-stored sediment (Table 2); the difference was significant ($p < 0.05$). The range of mortalities in this second test was virtually the same as in the first test. Had the first test been conducted with an additional low-level test concentration, the LC50s of both tests probably would have been the same.

The LC50s of the Mercer Lake sediment spiked with DDT also indicated decreased toxicity when the sediment was frozen (Table 2). The LC50 in the cold-stored sediment, 11.1 μg/g, was significantly different ($p < 0.05$) from the value of 23.2 obtained in the frozen-stored sediment. No LC50s could be calculated from the water column data in the DDT tests because of concentrations lower than detection limits (Table 2).

The three endrin tests did not exhibit the same toxicity relationships between cold-stored and frozen-stored sediments (Table 2). The endrin test with Soap Creek Pond sediment showed decreased toxicity in the frozen sediment, as did the DDT tests. The LC50s were 5.1 μg/g in the cold-stored sediments and 7.7 μg/g in the frozen-stored sediment. The difference was significant ($p < 0.05$), although the 95% confidence intervals were close.

Both remaining endrin tests, conducted with Mercer Lake sediment, indicated no significant difference ($p > 0.05$) in toxicity between cold-stored and frozen-stored sediment. The respective LC50 values for cold- and frozen-stored sediment for the first of these tests were 19.6 and 21.7 μg/g and for the second test, 10.3 and 9.8 μg/g. The LC50 values in the first test were about twice as large as those in the second test. The cause of this increase in toxicity between tests is unknown, but it may have been related to a change in the bioavailability of endrin in the sediment.

Water LC50s for the endrin tests followed the pattern of the sediment LC50s (Table 2). In the endrin test with Soap Creek Pond sediment, a decrease in toxicity was indicated for frozen-stored sediments. The LC50 for soluble endrin was 1.8 μg/L in the cold-stored sediment test beakers and 3.6 μg/L in the frozen-stored sediment test beakers, a significant difference ($p < 0.05$). LC50s based on total endrin for the test were very similar to those based on soluble endrin.

The water LC50 values for the two endrin tests conducted with Mercer Lake sediment showed no significant difference ($p > 0.05$) in toxicity between cold-stored and frozen-stored sediment beakers. The values for soluble endrin for cold- and frozen-stored sediments were 3.6 and 3.9 μg/L, respectively, in the first test, and 1.4 and 1.8 μg/L, respectively, in the second test.

Water TOC levels at the end of the tests were generally higher in the beakers containing cold-stored sediment than frozen-stored sediment, averaging 7.1 and 3.9 mg/L, respectively (Table 3). These higher levels were most likely related to the less consolidated cold-stored sediment particles that *Hyalella* kept in suspension. In contrast to the water TOC levels, soluble organic carbon (SOC) concentrations averaged 2.5 mg/L in the cold-stored sediment test beakers and 3.1 mg/L in the frozen-stored sediment test beakers. The SOC was significantly higher ($p < 0.05$) more than 70% of the time in the frozen-stored sediment test beakers, as compared with the cold-stored sediment test beakers, and was presumably released from the sediment by freezing. Sediment microorganisms and macroinvertebrates killed during the freezing process might contribute to the release of carbon upon thawing. Both TOC and SOC in the water column were generally lower from Soap Creek Pond No. 7 sediments than from Mercer Lake sediments (Table 3). TOC values from cold-stored sediment beakers averaged 4.9 and 8.5 mg/L, respectively, for Soap Creek Pond and Mercer Lake sediments. Frozen-stored sediment beaker TOC values averaged 3.6 and 4.2 mg/L for the two sediments. SOC values for cold-stored sediment beakers averaged 1.9 and 3.0 mg/L, respectively, for Soap Creek Pond and Mercer Lake sediments, while the frozen-stored sediment beakers averaged 2.6 and 3.6 mg/L, respectively.

The higher levels of SOC in the water of the DDT frozen-stored sediment beakers is assumed to have been a factor in reducing the toxicity. The ability of dissolved organic matter to reduce the bioavailability of organic contaminants has been documented by Landrum et al. [19,20].

The lack of decreased toxicity in the frozen-stored sediment beakers in two of the endrin tests conducted with Mercer Lake sediment in the presence of elevated SOC levels may be related to the carbon partitioning relationships for endrin. The partition coefficient for DDT based on organic carbon content (K_{oc}) is 4×10^6, while that of endrin is only 1.7×10^3. Similarly, the octanol/water partition coefficients (K_{ow}) for the two chemicals are 8×10^6 and 3.5×10^3, respectively [21]. The de-

Table 3. Organic carbon levels in water column at end of 10-d tests using cold-stored and frozen-stored sediments

Test	Initial measured pesticide concn. (μg/g dry solids)	Organic carbon in water (mg/L; $n = 3$)			
		Total ($x \pm$ SE)		Soluble ($x \pm$ SE)	
		Cold	Frozen	Cold	Frozen
DDT					
Soap Creek Pond No. 7	<0.4	6.7(0.3)	4.0(0.3)	1.4(0.4)	2.3(0.4)
	9.6	5.6(0.4)	3.9(0.3)	2.4(0.1)	3.1(0.2)[a]
	16.1	6.5(0.7)	5.8(1.5)	2.4(0.2)	2.9(0.2)
	44.8	3.5(0.2)	3.9(0.05)	2.4(0.1)	2.8(0.1)[a]
	83.5	3.7(0.1)	5.9(1.3)	2.6(0.05)	4.1(0.3)[a]
	194.8	2.8(0.4)	2.4(0.1)	2.0(0.2)	2.3(0.1)
Soap Creek Pond No. 7	<0.4	–	4.2(0.1)	2.0(0.02)	3.1(0.02)[a]
	1.8	–	2.9(0.03)	1.3(0.1)	2.6(0.03)[a]
	9.6	–	3.6(0.1)	2.5(0.03)	3.4(0.03)[a]
	16.1	–	4.3(0.2)	2.6(0.1)	3.4(0.02)[a]
	44.8	–	4.0(0.1)	2.6(0.1)	3.4(0.1)[a]
	83.5	–	4.1(0.03)	2.8(0.1)	3.4(0.1)[a]
Mercer Lake	<0.7	9.8(0.6)	3.0(0)	1.6(0.02)	1.2(0)
	1.3	10.3(0.2)	3.4(0.1)	2.1(0.05)	2.9(0.1)[a]
	8.0	6.7(0.3)	3.1(0.1)	1.6(0.1)	2.1(0.1)[a]
	20.0	5.7(0.1)	4.2(0.1)	2.3(0.1)	3.2(0.1)[a]
	71.3	3.4(0.1)	3.9(0.04)	1.9(0.1)	3.0(0.1)[a]
	160.0	3.1(0.2)	3.0(0.2)	2.0(0.1)	2.1(0.04)
Endrin					
Soap Creek Pond No. 7	<0.4	10.1(0.1)	3.4(0.04)	1.3(0.01)	1.9(0.01)[a]
	3.0	3.9(0.1)	2.9(0.1)	0.7(0.2)	1.5(0.04)[a]
	8.7	10.1(0.02)	2.7(0.1)	1.4(0)	2.2(0.01)[a]
	19.6	3.1(0.1)	2.7(0.03)	1.4(0.1)	2.1(0.1)[a]
	40.4	1.2(0.05)	2.0(0.1)	1.1(0)	1.4(0.1)
	62.1	1.6(0.1)	1.8(0.1)	1.0(0.02)	1.4(0.02)[a]
Mercer Lake	<0.7	18.6(0.5)	4.0(0.5)	2.5(0.1)	3.9(0.1)[a]
	2.0	8.4(2.0)	5.5(0.2)	3.7(0.1)	4.5(0.1)[a]
	5.3	6.1(0.8)	4.8(0.1)	3.8(0.1)	4.3(0.2)
	13.3	6.6(0.2)	4.6(0.1)	3.7(0.05)	4.2(0.1)[a]
	13.3	9.4(0.1)	4.7(0.01)	3.8(0.05)	4.8(2.0)
	–	6.2(0.3)	4.2(0.1)	3.6(0.2)	4.1(0.1)
	100.0	4.1(0.1)	5.1(0.6)	3.5(0.1)	3.3(0.1)
	266.7	5.0(0.3)	4.1(0.1)	5.0(0.1)	4.0(0.05)
Mercer Lake	<0.7	17.9(0.4)	4.1(0.1)	2.5(0.1)	3.4(0.1)[a]
	1.3	12.3(0.4)	4.1(0.04)	2.9(0.1)	3.6(0.1)[a]
	1.3	13.8(0.9)	4.5(0.01)	3.0(0.1)	3.9(0.4)[a]
	8.0	12.5(0.8)	5.0(0.02)	3.3(0.1)	4.6(0.05)[a]
	20.0	6.3(0.3)	4.8(0.1)	3.8(0.1)	4.7(0.1)[a]
	66.7	3.6(0.02)	3.8(0.1)	3.0(0.05)	3.6(0.1)[a]

[a]Soluble organic carbon from frozen sediment significantly greater than that from cold sediment ($p < 0.05$).

creased adsorptive capacity of carbon for endrin as compared with DDT could thus have affected their relative toxicities.

Nebeker et al. [6] studied the effect of sediment organic carbon on the toxicity of DDT and endrin to *Hyalella*. They reported a decrease in toxicity with increasing sediment organic carbon content for DDT and no effect on toxicity with increasing sediment organic content for endrin. Sediments with higher concentrations of TOC have a greater capacity to adsorb nonpolar organic compounds, thereby reducing their toxicity [5]. The present tests indicate a decrease in DDT toxicity with increasing sediment organic carbon content for both cold-

stored and frozen-stored sediments (Table 2). The present tests also indicated a decrease in toxicity with increasing sediment organic carbon for endrin-spiked cold-stored sediments. For frozen-stored sediment, however, the first endrin test run with Mercer Lake sediment (11% TOC) indicated a decrease in toxicity and the second test (11% TOC) indicated no difference in toxicity, as compared with the endrin test run with Soap Creek Pond sediment (3% TOC).

The endrin-spiked sediments from Soap Creek Pond and the second Mercer Lake test in the present study were from the same lots previously studied by Nebeker et al. [6]. There were essentially no changes in toxicity in the Soap Creek Pond sediments and only a slight decrease in toxicity in the Mercer Lake sediments over approximately nine months. While the DDT-spiked sediments from Soap Creek Pond used by Nebeker et al. [6] were prepared mostly at the same time as the Soap Creek Pond DDT-spiked sediments in the present study, they were not from the same lots. After approximately 19 months, the sediments used in the present study were more than twice as toxic, as reflected by LC50 level, as the sediments used by Nebeker et al. [6]. These results may suggest a possible increase in toxic breakdown products of DDT and the relative stability of endrin toxicity over time.

Manipulations that alter the physicochemical relationships of a sediment prior to toxicity testing or other analyses should be avoided. Data from studies using cold-stored and frozen-stored sediments may not be comparable. The freezing of sediments to be used for biological testing or freezing as a storage procedure is not recommended.

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